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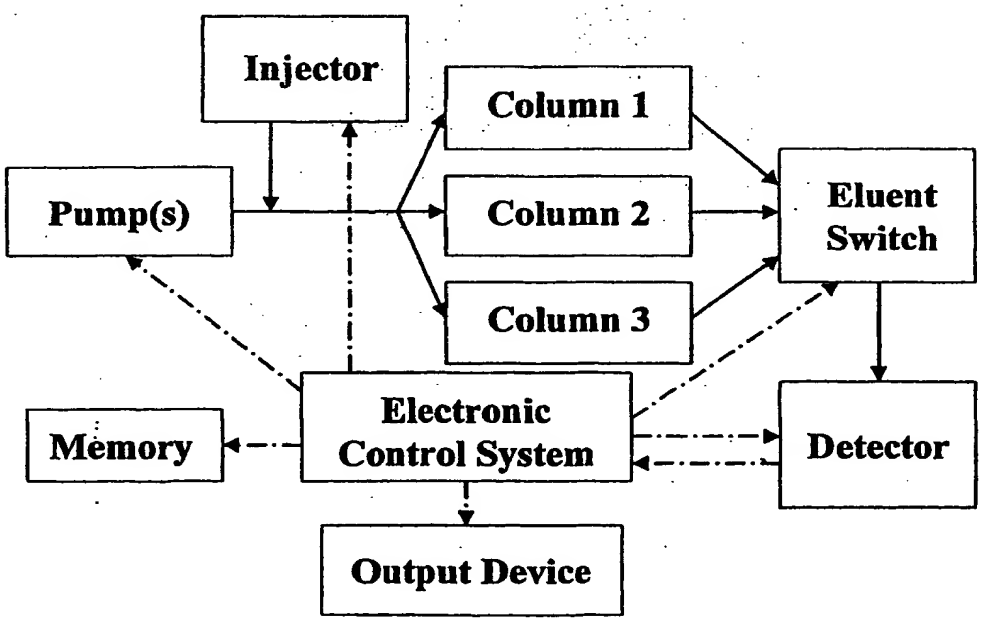


(57) Abstract

This invention relates to a high efficiency chromatographic system. More specifically, the present invention relates to a chromatographic system for determining the physicochemical properties of one or more compounds using at least tw chromatographic units in eluent flow communication with one eluent analyzer via an intermediate eluent switch. The present chromatographic system allows determination of physicochemical properties through the use of multiple chromatographic units in communication with one eluent analyzer via an eluent switch.

Chromatographic System with Pre-Detector Eluent Switching

Field of the Invention

This invention relates to a high efficiency chromatographic system. More specifically, the present invention relates to a chromatographic system for determining the physicochemical properties of one or more compounds using at least two chromatographic units in eluent flow communication with one eluent analyzer via an intermediate eluent switch.

Background and Summary of the Invention

The emergence of automated chemical synthesis platforms coupled with combinatorial techniques as a routine tool in the pharmaceutical industry has enabled the synthesis of large numbers of molecules in a relatively short time. Millions of potential new drug candidates are created every year, and both pharmaceutical and biotechnology industries have embraced the challenge in recent years of developing new, faster and more efficient ways to screen pharmaceutical compounds in order to rapidly identify "hits" and develop them into promising lead candidates. This has created the need for high-throughput analytical approaches to characterize the synthesized compounds and has prompted the development of chromatographic systems specifically designed for the automated high-throughput identification, purity assessment or purification of combinatorial libraries.

Currently, automated, semi-quantitative assessment of combinatorial libraries is most readily accomplished by coupling HPLC with UV detection and mass spectrometry. Rapid HPLC methods with columns capable of delivering high-resolution separations have been developed in recent years, and have been well received by the drug discovery industry as a powerful tool particularly suited to handle the expanding analytical needs of combinatorial chemistry. The ability to characterize chemical libraries derived from combinatorial synthesis has in turn revealed that the purity of the compounds generated by this method is not necessarily high enough for biological evaluation of these compounds. Consequently, the scope of the high-throughput HPLC techniques initially designed and developed for structure

delivered to each column from a single HPLC pumping system, the flow from the pump splitting evenly between the columns (provided that the columns have comparable back pressures). Kassel et al. modified the IonSpray interface of the system to support flows from multiple columns and the eluents of the two columns were simultaneously introduced into the IonSpray source housing, and analyzed by mass spectrometry. This particular configuration allows the purification of chemical libraries based on mass spectrometry signal-detected fraction collection. Prior to performing the chromatographic separation, the mass and position of the expected products synthesized in the microtiter plate wells are specified. When a particular compound is detected by mass spectrometry in the course of the HPLC elution, the fraction collector connected to the column from which the compound is eluting is triggered, and the sample is collected in a specific tube determined by the position of the autosampler (for example, if the sample is drawn from well 1 of the autosampler/synthesis rack, the sample will be collected into tube 1 of the fraction collector rack). Thus, only compounds matching the molecular weight of the desired products are collected, and only one fraction is collected for each sample injected.

A major limitation of Kassel et al.'s parallel LC/MS technique is that the products to analyze must be of unique mass: false triggering of the fraction collectors is observed if two eluted compounds are of the same mass and similar ionization response. Thus, the synthesis of the combinatorial libraries must be carried out with the added restriction that no two expected products should yield the same molecular weight products. Further, for the flow to be equivalently transferred to the two columns requires that they have comparable back pressures because delivery of the solvent gradient is performed by a single pumping system. This generally requires that the columns be of the same size and be packed with the same chromatographic material. In addition, the design also dictates that both columns are eluted with identical mobile phase compositions. This limitation is usually of no consequence for the purification/purity assessment of combinatorial libraries, since the synthesized compounds are generally structurally related and exhibit similar chromatographic behaviors.

The Kassel et al. system is particularly well suited for one of the major challenges found in the pharmaceutical industry: high-throughput structure

denied access to the MS capillary inlet thus hits the outside wall of the inner source cylinder. The stream of heated dry nitrogen gas facilitates evaporation of the solvent in the atmosphere. Although the maximum flow from each electrospray channel is small (100μL/min) this potentially constitutes a health hazard, depending on the nature of the mobile phase or the analyte.

With the advent of combinatorial chemistry and the need to develop assays for the large numbers of compounds being made available using that technology, many researchers have focused their efforts on developing in vitro tests/assays that provide biologically significant compound information. Much work has been directed to the correlation of certain physicochemical properties with biological activity, both in the search for new therapeutic agents and in the understanding of compound toxicity from medicinal and environmental perspectives. For example, physicochemical properties of recognized significance to evaluation of a compound's biological activity are its lipophilicity, hydrophilicity, interfacial pKa, and membrane affinity, among others. The determination of these properties is critical for QSAR studies, and the worldwide discovery effort. The present invention relates to a system for determining not only chemical structure, but also the physicochemical properties critical for such QSAR studies and drug discovery efforts. The chromatographic process represents a reversible equilibrium of solutes between the mobile phases and the stationary phases. The magnitude of solute retention is a direct result from this equilibrium and is typically expressed by a parameter, the capacity factor, $k'=(t_r-t_o)/t_o$ where t_o is the dead time and t_r is the retention time of the solutes. The capacity factor is therefore a stoichiometric mass distribution equilibrium of solutes between the mobile phases and the stationary phases, and its determination allows the calculation of various physicochemical values according to pre-determined algorithms.

The distinction between serial and parallel column chromatography is important. Serial column chromatography is an established method and involves automatically changing columns after a chromatographic run. This allows multiple columns to sequentially be evaluated. In contrast, parallel column chromatography allows multiple columns to simultaneously access one detector. Parallel

columns, capillary electrophoresis chromatography (CEC) columns, Gas Chromatography (GC) columns, super-critical fluid columns and microchips. The eluent analyzer unit is any instrument capable of identifying the presence, physicochemical characteristics and/or chemical structure of a compound, including (but not limited to) a mass spectrometer (MS), a Fourier transform infra red spectrometer (FTIR), a Fourier transform ultra violet spectrometer (FTUV), standard UV detector, fluorescent detector, electrochemical detector, refractive index detector and a Fourier transform nuclear magnetic resonance spectrometer (FTNMR).

Brief Description of the Drawings:

Fig. 1 depicts a diagram of one embodiment of the high-throughput chromatographic system described in this invention.

Fig. 2 depicts the hardware requirements and associated connections of the two 13-port valves contained within one embodiment of the injection system.

Fig. 3 depicts the configurations of the two valves of the injection system in loading mode (position 1) and injection mode (position 2).

Fig. 4 depicts Kassel's model for the simultaneous introduction of the column eluents into the electrospray source housing of a four-column HPLC/MS system.

Fig. 5 is a schematic diagram of a four-column High Throughput HPLC system equiped with an eluent switch device.

Fig. 6 depicts the hardware connections of the pre-detector four-port switching valve system.

Fig. 7 depicts a schematic diagram of a prototype two-column eluent switch system. As shown, only two manifold inlets (1&2) are used for the two-column prototype. The remaining inlets (3&4) may accommodate two additional columns.

Fig. 8 details elution of 4-methylanisole from an ^{Ester}IAM.PC$^{C10/C3}$ column, a C8 column, and from both columns in parallel.

Fig. 9 details elution of 4-methylanisole from ^{Ester}IAM.PC$^{C10/C3}$ and C8 columns using the eluent switch of Fig. 7 and one pump for both columns.

Fig. 10 details data obtained using the eluent switch of Fig. 7 in a one-pump/two-column set up.

switching device immediately upstream (eluent flowwise) from the eluent analyzer. For purposes of this invention, "eluent analyzer" and "detector" are synonymous.

In one apparatus embodiment, the present invention is directed to a chromatographic system comprising: at least two chromatographic units each having a sample compound loading system, a mobile phase entry port, an eluent exit port, and a stationary phase; a mobile phase supply system for delivering mobile phase to the mobile phase entry port of each chromatographic unit; a detector having an eluent sampling port, this detector capable of providing a signal of the presence or identity of an eluted sample compound in an eluent sample delivered to the sampling port; an eluent switch in eluent flow communication with each of the chromatographic units for delivering aliquots of eluent from each chromatographic unit sequentially to the eluent sampling port on the detector; and a data management device for receiving or storing signals from the detector.

The mobile phase supply system of this embodiment optionally includes a mobile phase pump for each chromatographic unit. In this embodiment, the pressure generated by said pumps can be constant or varied to control the flow rate of the mobile phase in each chromatographic unit. In another optional variation of this embodiment, the sample compound loading system of this embodiment comprises a valve that allows delivery of sample compounds and mobile phase into the chromatographic units when the valve is in one position and allows delivery of only mobile phase into the chromatographic units when the valve is in a second position.

In another apparatus embodiment, the present invention is directed to a system comprising: a fluid supply system; a compound loading system including multiple discreet chambers for receiving unique compound samples, said chambers in fluid flow communication with said fluid supply system; a detector having a sampling port, the detector capable of providing a signal of the presence or identity of a sample compound delivered to the sampling port; a sampling switch in fluid flow communication with the sample chambers and sampling port for delivering aliquots from each sample chamber sequentially to the sampling port on the detector; and a data management device for receiving or storing signals from the detector.

In an optional variation of this embodiment, the compound loading system of this embodiment comprises a valve or set of valves that allows delivery of

wherein the eluent from each of the chromatographic units is directed into an eluent switch adapted to deliver sequentially portions of the eluent from each of the chromatographic units comprising: applying the sample compounds into at least two chromatographic units each having a sample compound loading system, a mobile phase entry port, an eluent exit port, and a stationary phase; supplying mobile phase into the mobile phase entry port of each chromatographic unit using a mobile phase supply system; eluting the sample compounds from each of the chromatographic units into an eluent switch in eluent flow communication with each chromatographic unit; said eluent switch being capable of sequentially delivering aliquots of eluent from each chromatographic unit to a detector in eluent flow communication with the eluent switch; detecting the presence or identity of an eluted sample compound in an eluent portion delivered to the detector; generating a signal when such presence or identity of an eluted sample compound is detected; and receiving or storing such signal.

In optional variations of this embodiment, the application of sample compounds to the chromatographic units is accomplished by using an injector comprised of a valve and at least two sample loops wherein the injector valve, when placed in one position, allows delivery of the compounds into the sample loops and also allows the mobile phase to bypass the sample loops and be delivered into the chromatographic units; said sample valve, when placed in a second position, allows delivery of the compounds and mobile phase from the sample loops into the chromatographic units. Further, the step of detecting the presence or identity of an eluted compound is optionally accomplished by using a detector comprised of a mass spectrometer, a Fourier transform infra red spectrometer, a Fourier transform ultra violet spectrometer, or a Fourier transform nuclear magnetic resonance spectrometer. Other optional variants of this embodiment include the step of eluting the sample compounds using mobile phase supplied to at least two chromatographic units comprised of high performance liquid chromatography columns, gas chromatography columns, super-critical fluid columns, capillary electrophoresis columns or chromatographic microchips.

By allowing simultaneous chromatographic runs using more than one chromatographic unit (while only requiring one eluent analyzer), the present chromatographic system provides certain advantages when coupled with methods for

value according to a pre-determined algorithm. Typically at least a portion of the compounds tested are compounds having a known value for the physicochemical characteristic being calculated. Preferably the method is implemented using liquid chromatography, more preferably high pressure liquid chromatography, to carry out the step of contacting each test solution with the surface. The stationary phase comprises the surface, the aqueous medium is the mobile phase, and the affinity-dependent parameter is the retention time for each compound.

One embodiment of this method (to be used in conjunction with the chromatographic system of the present invention) requires minimal experimental effort for the high throughput simultaneous determination of both ^{bulk}pKa and ^{surface}pKa of compounds. The method for determining pKa involves dissolving a set of compounds in a plurality of aqueous media, each having a unique pH to form a multiplicity of test solutions of said compounds (each also having unique pH). Each of the test solutions is then contacted with a chromatographic unit containing a surface exhibiting a compound-dependent affinity for the dissolved compounds, and then a parameter dependent upon the affinity of the surface in each solution for each of the compounds may be calculated after the compounds are evaluated by analysis of each solution after it is contacted with the surface. The dissociation constant for each compound is then calculated from the pH-dependent measured parameters for each respective compound.

The step of contacting each solution with the surface is carried out by liquid chromatography using a stationary phase and a liquid mobile phase wherein the stationary phase comprises the surface and the aqueous medium is the mobile phase. In such embodiments the test solutions having unique hydrogen ion concentrations are effectively formed in the chromatographic column with the aqueous medium mobile phase. The affinity-dependent parameter can be the retention time for each compound. The surface can be selected from a wide variety of commercially available chromatographic supports. One preferred class of surfaces is a membranous or membrane mimetic surface comprising phospholipids covalently bound to a solid substrate.

Chromatography will provide the capacity factors k' of each of the many compounds detected upon elution from the column, at (for example) various

varied for each respective chromatographic unit, while mobile phase, pressure and flow rate are the same for each. A test compound with known physicochemical properties is simultaneously injected into each chromatographic unit and eluted. Portions of the eluent from each column are sequentially delivered to the detector via the eluent switch. The detector determines the presence of the compound and delivers a signal from which the elution profile of each respective column is determined. Upon analysis of this data, conclusions concerning the interaction between the test compound and the various surfaces employed in the stationary phase are drawn. For example, the comparative non-specific binding of a known compound with various surfaces can be readily compared using the present invention.

In particular, when the chromatographic surfaces are membrane mimetic surfaces, the membrane affinity fingerprint (MAF) of said compound can be determined. Briefly, the membrane binding properties of test compounds can be calculated, or they can be determined empirically with use of, for example, liposomes, immobilized artificial membranes (such as those described in U.S. Patent 4,931,498, the disclosure of which is incorporated herein by reference), Langmuir Blodget films, computer chips or similar devices with immobilized lipids, capillary zone electrophoresis columns coated with membrane lipids, and the like. In the case of immobilized artificial membranes (IAMs), the numerical values characteristic of membrane affinity are determined chromatographically using an aqueous mobile phase and a stationary phase comprising a membrane mimetic surface as defined in U.S. Patent 4,931,498. Membrane binding properties of a set of test compounds of unknown biological activities are compared to the membrane binding properties of control compounds having known *in vivo* biological activity to assess the probability that the test compounds will exhibit one or more biological activities *in vivo*. For each control compound there is a defined and ordered set of numerical values characterizing a biologically relevant interaction (e.g., affinity) of that compound with each of the selected membrane mimetic surfaces.

The ordered set of numerical values for each control compound or each set of control compounds (i.e., a "training set") can be represented by the expression $<C_1, C_2, ..., C_n>$ wherein n is the number of membrane mimetic surfaces identified and used in the screening method. A similar ordered set of numerical values $<T_1, T_2, ...,$

having the predetermined characteristic (to be used in conjunction with the present chromatographic system) comprises subjecting the compound mixture to a separation process using a first set of compound separation parameters (associated with the first chromatographic unit) to at least partially separate the compounds in the mixture into a series of separation variable-dependent fractions (Fa) in the order Fa_1, Fa_2, Fa_3,... Fa_n wherein n is the number of fractions collected using the first set of separation variables, at least a portion of which fractions include one or more compounds of the compound mixture. In step (b), step (a) is repeated for another chromatographic unit that has a second unique set of separation parameters to produce a second series of separation parameter-dependent fractions (Fb) in the order Fb_1, Fb_2, Fb_3,... Fb_m wherein m is the number of fractions collected using the second set of separation parameters. Thus, for each chromatographic unit used, a set of fractions is generated. Each of the qth fractions obtained using each set of separation parameters (wherein q is the respective order number of the fractions obtained using each set of separation parameters) is combined to provide combined qth fractions.

Spectral data is then obtained on a sample of each of said combined fractions, the data being characteristic of the compound or compounds in the combined fractions. Each combined fraction is analyzed to detect the presence of the predetermined chemical, physical or biological characteristic and identifying each of those combined fractions that exhibit the predetermined characteristic. From this, a comparison of the spectral data for each of the combined fractions exhibiting the predetermined characteristic to identify spectral data common to each of said combined fractions can be made, and the compound or compounds characterized by the spectral data common to the combined fractions exhibiting the predetermined characteristic in said combined fraction can be identified.

In another embodiment, compound analysis is performed on the eluted sample compounds to determine their chemical structure. For example, compounds with predetermined physicochemical properties can be identified using the present invention. The present invention provides an embodiment wherein a library of structurally related compounds is eluted from two or more chromatographic units. Aliquots of the eluent from each column are delivered to a detector capable of determining both the presence and the structural identity of the eluted compounds.

exemplified herein, the design can be adapted to include more or less than four columns. The columns can be simultaneously loaded with the same test solution containing a set of compounds dissolved in a solvent mixture, and run in parallel at constant flow rates. Delivery of the mobile phase by four independent pumping systems is crucial if a constant predetermined eluent flow rate is to be run through each column which contains different packing materials, as required by the mass spectrometer interface. If a single pump was to deliver solvent to a set of columns such that they contain different packing material, the difference in back pressure exhibited by said columns would result in the mobile phase being unequally split (non-similar flow rates) onto the columns.

For any one column, the back pressure is generally a function of the length of the column, the particle size and shape of the chromatographic material, and the viscosity of the mobile phase. Therefore, unless strictly identical columns are used in the HPLC system, the use of four independent pumping systems is imperative to ensure constant flow rates throughout the columns.

The advantage of using dedicated pumps for each column, as opposed to a single pumping system delivering the mobile phase to all columns (or using no pump at all), is that it allows the control of the flow rates throughout the columns. A system using a single pump would require the implementation of additional hardware to compensate for the undetermined flow rates in each column. For example the individual flow rates would have to be measured, and the development of the software for data processing would require more effort to account for the fact that the analytes on each column would not elute under comparable conditions (uneven flow rates). In addition, flow dependent experiments would be impossible to run, and quality control would be significantly affected, since it would be difficult to determine the amount of sample actually loaded on each column. With a one pump/one column system however, control of the flow rate is ensured, and thus no correction for k' is necessary. Multiple flow rates can be tested, and quality control is maximized.

In one embodiment, the injection device is such that it allows loading of the test solution onto one, two, three or all four columns. The injection system may comprise four individual injectors (one per column), which are loaded manually, and are electronically controlled so that simultaneous or staggered injection of the sample

the chromatographic columns, although other types of detectors are possible. The analytes eluting from the columns will alternatively be introduced into the electrospray source housing with the help of a pre-detector valve system incorporated between the columns and the mass spectrometer electrospray chamber.

In one embodiment of the invention, the eluent switch device contains a set of four four-port switch valves controlling the destination of the eluent: toward the mass spectrometer for analysis or to the waste container (or fraction collector). One valve of the eluent switch is in fluid communication with one individual chromatographic column, and after passing through the valve, the eluent from each column is transferred into a pre-detector manifold before entering the detection system. This manifold is also connected to a "wash solvent" outlet (controlled by another four-port switch valve) allowing the introduction of a reference solvent mixture into the manifold after every column eluent analysis. This would minimize sample carryover and systematic error due to contamination. If desired, when the detection system is an MS, the wash solvent may contain a reference compound (R*) of known mass and ion current signal allowing easier data processing and interpretation: the mass spectral data obtained from each individual column would be separated by an identical signal corresponding to the reference compound.

In one embodiment, the pre-detector eluent switch device is electronically controlled and allows the operator to preset the time, sequence and/or volume parameters for the introduction of the eluent from the various columns to the eluent analyzer. For example, the analysis sequence can be set so that eluents from column 1, column 2, column 3 and column 4 alternatively enter the electrospray housing for 2-3 seconds at a time, with introduction of reference solvent in between analyses for sample carryover problem elimination. Thus, the eluting samples from each column are analyzed alternatively, according to a pre-determined sequence programmed by the operator through a computer software interfaced with the LC/MS system. Each fraction is analyzed and its composition determined, so that each compound identified (MS) is associated with a retention time (chromatography) and a capacity factor. The data is processed, sorted and stored as four independent data files. From each capacity factor, the calculation of various physicochemical values for each compound detected is performed according to pre-determined algorithms. An

controllable components include the mobile phase supply system, the injector or sample compound loading system, the eluent switch, and the detector. Such electronic control is achievable for any of the components individually or may be used to coordinate the control of the components simultaneously. An example of such electronic control systems is a computer with software.

The detector or eluent analyzer of the present invention sends a signal in response to the presence of a sample compound. This signal is received or stored by a data management device, which either stores the signal in memory or transforms the signal into a readable form of data and sends the data to an output device. This data management system may be the same device as used to control the operation of the various components of the system (the electronic control system) or may be a separate, independent device from the electronic control system. Such data management control systems are known in the art.

As shown in Fig. 1, one embodiment of the proposed technology is based on a chromatographic system containing a pumping system, an injector, multiple columns (preferably four, although a higher number is possible) run in parallel, a pre-detector eluent switch module, and a detector which must be such that it allows identification and quantification of detected compounds alone or in mixtures with other compounds on a millisecond time scale. An HPLC is the preferred chromatographic support for this invention, although other chromatographic techniques are suitable for the purpose (*e.g.*, CEC, microchips, GC). The preferred detection device is a mass spectrometer, although other detection systems, such as FTIR, FTUV and FTNMR detectors are acceptable. The use of a tandem mass spectrometer (MS/MS) may be required for more complex cases where the identification of the compounds eluting from the columns is not possible after a single mass spectrometric analysis. Chemometrics is used to analyze data derived from the technologic design described in this invention.

The injection system may allow sequential loading of sample solutions onto the columns (one column-one sample) or simultaneous injection of the same sample onto several columns (the number of columns to be used in a run is determined and set by the operator prior to starting the experiment via a computer software interfaced with the HPLC/MS system).

of HPLC. These sample injectors should be electronically operated, which ensures the injection being conducted simultaneously. Having electronic control of the injection valves would also allow the user to program the multiple injections at different time intervals (staggered instead of simultaneous), should such an experimental design be required.

In the case of simultaneous sample loading on several columns, one design for the sample injector of the present invention is shown in Figs. 2 and 3. The interface between the injector and the multi-column unit is also illustrated in Fig. 2. Two head-to-head thirteen-port valves (valves A and B), connected with different sample loops and microtubes, make up the injection system for simultaneous loading of a sample solution onto a selected number of columns. Sample loop-1 (S_1) is installed between port-1 of valves A and B along with an electronically controlled shut-off valve right before the loop to prevent loading of the sample onto this loop if desired. Similarly, sample loops S_2, S_3 and S_4 are installed between ports 4, 7 and 10, respectively, of valves A and B with a shut-off valve upstream from each loop. Pump-1 (P_1), dedicated to the delivery of mobile phase 1 to column-1 (C_1), is connected to port-2 of valve A. Similarly, pump P_2 (dedicated to column C_2), pump P_3 (dedicated to column C_3) and pump P_4 (dedicated to column C_4) are connected to ports 5, 8 and 11 of valve A, respectively. All columns are connected to valve B: C_1 to port-2, C_2 to port-5, C_3 to port-8 and C_4 to port-11. Ports 3, 6, 9 and 12 of both valves A and B function as bridges between the pumps and their corresponding columns (loading configuration) or between sample syringe and the open end for waste (injection configuration). The central ports of valves A and B are connected to the sample syringe and the waste container, respectively. The arrows indicate the direction that the sample solution or solvent will flow. The sample loops in our invention can be of the same size or have different loading capacities.

The configurations of these two valves are shown in Fig. 3. In the loading configuration, both valve A and B are in position 1. The sample is introduced through the injection port (central port of valve A) and is loaded onto the different sample loops between ports 1, 4, 7 and 10 of valves A and B. At this point, the sample loops, initially filled with mobile phase, are loaded with the sample solution. If the sample loops are identical and no air bubble is accidentally introduced in the course of

(Fig. 4B). Each sprayer has its own nebulizer gas supply, which delivers 40 psi N_2 to enhance the nebulization and evaporation of solvents. The system can be fully automated to switch between parallel (dual) analytical and parallel (dual) preparative LC/MS analyses. Optimization of the signals from the two parallel sprayers was accomplished by Kassel and coworker by adjusting the spacing between the spray tips and the X-, Y-positioning of the dual-electrospray device relative to the entrance aperture (orifice) of the mass spectrometer.

The methodology can be adapted to our parallel multiple-column HPLC/MS system by making sheath (quadruple) electrospray with the nebulizer gas supply in the outer most position. One of the concerns for this design is how well the nebulizer gas will function and reproduce on this system when the positions of nebulizer gas with each column are different.

The problem of eluent switching between multiple columns and one detector can be solved by implementing the design described below, which can be implemented with existing commercially available technology.

Fig. 5 shows one example of the eluent switching system: after each column, a four-port solenoid valve is used to switch the eluent from the MS detector to fraction collector or waste according to a signal sent by a computer program interfaced with the whole system.

The general advantage of solenoid valves is that the elution is continuous; consequently there is no disturbance on the flow rate, hence no disturbance regarding the capacity factor k'. Such valves are programmed for automatic operation, which can run for long hours to maximize the use of the mass spectrometer. The column-dedicated four-port valves used in this design are referred to as V_1, V_2, V_3 and V_4 for columns C_1, C_2, C_3 and C_4 respectively and their port connection and configurations are described in Fig. 6. The end of each column connected to port 2, and port 1 is connected to the electrospray ionization detector. Port 3 is connected to waste or fraction collection and port 4 remains unused. In position 1 the tunnels between ports 1 and 4 and between ports 2 and 3 are opened, so that the eluent from the column will go to waste or fraction collector. In position 2, port-1 and port-2 are connected, as well as ports 3 and 4, resulting in the eluent being delivered into the detection system. Electronic control (e.g., computer interface) of

Table 1

Quarter	Valve	Position
1	V_1	2
	V_2	1
	V_3	1
	V_4	1
2	V_1	1
	V_2	2
	V_3	1
	V_4	1
3	V_1	1
	V_2	1
	V_3	2
	V_4	1
4	V_1	1
	V_2	1
	V_3	1
	V_4	2

After passing through the column, the eluent from each column enters a flow splitter, which divides the mobile phase into two streams, typically to both waste/fraction collector and a switch valve. The function of the splitter is to prevent back pressure changes to the column when the flow is restricted by the switch valve, i.e., when the switch valve is off the mobile phase needs a path to flow to avoid creating back pressure to the column. The flow splitters thus allow control of solvent volume that goes to the detector, waste, or fraction collector, but equally important is that they prevent any back pressure changes to the columns during the cycling of the switch valve. Preventing oscillating back pressure to the columns while the switch cycles should prevent column lifetime from changing compared to conventional single

limited life-times of rotor valves limit their use in designing parallel chromatography equipment that will cycle > 86,000 times (at 1 Hz) over a 24 hour period. In addition to extended lifetimes, the rebuilding cost for the diaphragm valves is significantly less. Only the diaphragm itself needs to be replaced at very low cost. Diaphragm replacement should be infrequent; continuous operation for 2 weeks was achieved without any detectable loss of valve function. Note that since each column-valve unit is separately connected to the manifold, adding or removing columns from the device is simple, i.e., this flexible-manufacturing feature of the design allows the user to configure any number of columns.

Summary of Preliminary Experimental Results Using The Two-Column Eluent Switch System Shown in Fig. 7:

I. Phosphate buffer solution

The Phosphate Buffer Saline (PBS) solution was prepared by dissolving 0.2g of potassium phosphate monobasic, 1.15g of sodium phosphate dibasic and 2.922g of sodium chloride in 1 L of distilled water. This produced a PBS buffer with a 0.01M phosphate, 0.067M salt and pH around 7.5.

II. LC/MS conditions

The LC/MS system comprised a Hewlett Packard HPLC (HP 1100 series) interfaced with an Esquire_MS spectrometer. The HPLC had two binary pumps and a Diode Array detector (DAD). The mobile phase was a 0.01M PBS solution with 15% Acetonitrile and the flow rate was set up to 1 ml/min unless otherwise indicated.

III. Testing the eluent switch without columns

Preliminary studies were performed to test the operation of the switch valves in different solvents. This was necessary because this is the first time this type of valve, which has been designed for use with gas chromatography, had been used for liquids (Valco, personal communication). These preliminary studies were intended to show that the valve could function for weeks in typical mobile phases (buffers with and without organic modifiers). Acetonitrile, water, and 0.01M phosphate buffered saline

Table 2: ^{Ester}IAM.PC$^{C10/C3}$ and C8 chromatographic data for 4-methylanisole.Chromatograms are shown in Fig. 8.

	Columns					
	^{Ester}IAM.PC$^{C10/C3}$			C8		
	t_r (retention, min)	k' (Capacity factor)	$\omega_{0.5}$ (peak width)	t_r (retention, min.)	k' (Capacity factor)	$\omega_{0.5}$ (Peak width)
No Switch (Single column chromatography)	3.31	7.48	0.46	15.85	23.04	1.57
Eluent Switch (parallel chromatography)	3.17	7.13	0.44	16.3	23.71	1.35
Percent Change	5.1	4.7	4.3	2.8	2.9	14

When 4-methylanisole eluted (t_r ~ 16.5 min) from the C8 column (Fig. 8B), mobile phase from the IAM column (Fig. 8A) does not contain 4-methylanisole. Thus at 1 Hz eluent switching, the expected chromatograms should contain intervals of no absorbance from the IAM mobile phase (~ 1 sec), followed by absorbance from 4-methylanisole (~ 1 sec) from the C8 column. In other words, when eluent switching is sufficient to eliminate mixing between the mobile phases, oscillation is expected because the mobile phase from the IAM column is effectively a wash cycle that should drive the signal to baseline.

Note that the peaks in Fig. 8C do not show oscillations as the peaks elute (i.e. signal returns to baseline). This indicates that there is significant mixing in either (i) the manifold (~ 1 - 2 μL dead volume), (ii) post manifold tubing (1-2 μL dead volume), (iii) the UV detector cell (~14 μL dead volume), (iv) an unknown source, or some combination of (i), (ii), (iii), and (iv). An important concept is that when the flow rate is 1 mL/min, ~ 17 μL/sec flows through the system. At 1 Hz (sec) per channel, 17 μL of mobile phase elutes though the system and this volume of mobile phase should have been sufficient to wash components (i), (ii), and (iii). It is essential that these common post column conduits, shared by both column eluents, are washed. In other words, to validate the prototype design (Fig. 7) it was essential to

Liquid chromatography equipment interfaced with mass spectrometers is typically utilized with a UV detector. Consequently, we evaluated the eluent switch on a Bruker Esquire mass spectrometer equipped with a photodiode array detector (PDA). For these studies Estazolam (high ionization efficiency) was substituted for 4-methylanisole (low ionization efficiency). Fig. 10 compares 2 sec/column (Fig.10A) and 4 sec/column (Fig. 10B) eluent switch rates for Estazolam eluting from an ^{Ester}IAM.PC$^{C10/C3}$ and ^{Ester}IAM.PS$^{C10/C3}$ columns (synthesized in-house at CPBD) using a single pump to deliver the mobile phase at 2 mL/min., which equally splits between the two columns of identical size, particle size, shape and porosity. For this experiment 4 sec/column at 1 mL/min was not quite enough to bring the oscillations to baseline; a slightly higher rate would be needed. Nevertheless, Fig. 10 demonstrates that excellent peak characterization occurs when the system is washed.

V. Analyte calibration curves can be obtained from two columns and column stability does not change.

Calibration curves are essential for parallel chromatography to be used for quantitative analysis. Calibration curves and column stability data were generated for 4-methylanisole on ^{Ester}IAM.PC$^{C10/C3}$ and C8 columns using UV detection with an HP1100 HPLC system and the manifold switch shown in Fig. 7. It was shown that calibration curves are not affected by the eluent switch. In addition, column life times changed little to none when columns were modulated with the eluent switch. After ~ 8000 column volumes, less than 5 % change in *k'* occurred on the IAM column whereas there was little change on the C8 column.

Other embodiments of this design are identical to the hardware shown in Fig. 7 with the modification that 2 additional units can be added. Another modification is that an extra switch valve can can be plumbed to a 6-port manifold for washing purposes. Thus, four of the six manifold ports can be plumbed to 4 different columns. Of the two remaining manifold ports, one can be used to send eluent to the detector and the other manifold port can be used to introduce a wash cycle between columns.

In another embodiment of this design the flow splitter (Fig. 7) is a pressure release valve, which functions as a flow controller. Such modification is to

adapter that may be assembled directly onto the detector cell, provided minor modification of said detector cell. This would allow significant reduction of the dead volume created by the tubing connecting the manifold and the detector, thus minimizing band broadening effects. In addition, this would reduce the wash cycle time, and therefore would enhance resolution in data acquisition. Alternatively, the "detector cell" may be designed and/or manufactured so that it has multiple inlets to accommodate for multiple incoming LC eluent lines (for example four lines for each column eluent, and one for the wash solvent).

A 6-port manifold system requires a new controller device to be constructed. Such controller should operate the switch valves for each of the four columns and the timing of the wash cycle. A preferred controller will allow 1, 2, 3, or 4 columns to be used at any time. This goal can be acquired by having full computer control of the eluent switch valves. Valve switching can be actuated by TTL signals from an A/D board in a dedicated PC computer system. A software interface to the A/D board can be programmed using the LabVIEW computer programming language or other useful computer language. Such custom-made program can control the timing of the flow of each of the four columns and the wash cycle. In order to optimize performance of the system, the timing of each of the column cycles, as well as the wash cycle, can be controllable programmatically and can vary. Thus, column flow to the detector from different columns can be 0.5 sec for the first column 0.7 sec for the second, and any other value for the other columns. The wash cycle between columns is also variable.

Data Indexing Methods

Data from multiple columns can be stored into one data file only if unambiguous indexing of the signal from each column can be incorporated into the data file. An indexing method is needed to accomplish identification of each column's data in one data file.

For this purpose, a method for data acquisition by analysis of eluent streams from multiple chromatographic columns using an eluent stream analyzer that has a fluid sampling port and is capable of providing signals characteristic of detected chemical species is useful. An embodiment of this method comprises providing an

having a mobile phase input port and an eluent stream output port; (2) a mobile phase delivery system in fluid flow communication with the mobile phase input port, and (3) an eluent stream analyzer having a fluid sampling port and capable of providing signals characteristic of chemical species in a fluid received in said sampling port. To these components is attached an eluent switching valve assembly having (1) a fluid input port positioned for fluid communication with the eluent stream from each chromatographic column and with a source of at least one indexing fluid; (2) a fluid output port in fluid flow communication with the fluid sampling port of the eluent stream analyzer; and (3) a valve system for directing aliquot volumes of the respective eluent streams and indexing fluid in a programmed sequence through the fluid output port and toward the fluid sampling port on the eluent stream analyzer.

Typically, an electronic data storage device is used for receiving and storing signals from the eluent stream analyzer representative of analysis of eluent stream aliquots of each respective chromatographic column, and also included is a programmable controller (computer) including an algorithm including instructions for sensing the presence, volume or components of an indexing fluid aliquot at the fluid sampling port of the analyzer and directing signals from the analyzer for eluent stream aliquots corresponding to the respective chromatographic columns to electronic data storage registers/devices designated for data storage for the respective chromatographic columns.

In general, the multi-column chromatographic system for data indexing described above uses a fluid input port on the eluent switching valve assembly that is positioned for fluid communication with the eluent stream from each chromatographic column and with a pressure control source of at least one indexing liquid and at least one indexing gas. Analyzers useful for this method include, but are not limited to, mass spectrometers, infra-red spectrometers, nuclear magnetic resonance spectrometers, and ultraviolet spectrometers, fluorescence detectors, electrochemical detectors, and refractive index detectors.

One method to accomplish such identification involves using a wash cycle to index chromatography files for the high throughput HPLC instrument. For instance, a long wash pulse (no signal) at the beginning of the chromatographic experiment (i.e., before solute injection on any column in the high throughput HPLC

(and therefore the washing rates) are significantly different. The data in Fig. 11A is somewhat idealized in that it shows no change in the signal while data is being stored to a chromatogram. If this data is collected during the elution of a peak it may actually undergo a significant change. The software used in the present system must be able to distinguish between a rate of change characteristic of washing and that due to changing analyte concentration. This is possible since the rate of change from washing should be much higher. Fig. 11B shows data collected on a High-Throughput HPLC/UV (^{HT}HPLC-UV) instrument (the design of which is shown on Fig. 5, where the detector is a UV-Vis detector) using one of the available four channels (columns). The graph shows the elution profile (UV detection) for a sample of Warfarin (0.6 μg/μL in DMSO/PBS 30/70; injection volume: 20 μL) on an ^{ester}IAM.PS$^{C10/C3}$ column (4.6×30 mm; 15% acetonitrile in PBS, 1 mL/min). The eluent switch rate was set at 5 seconds per switch. The expanded view (Fig. 11B, right) of the signal around 150 sec is in agreement with the theoretical absorbance data profile shown in Fig.11A.

We initially focused on experiments that generated non-overlapping peaks (Figs. 8-10). Other tests were performed to verify that different wash cycles can be used to index and deconvolute chromatographic data files containing data from multiple columns. The hardware design used allows for each column and wash cycle to be cycled in any variety of order.

Fig. 12 shows the single elution of 4 different drugs from 4 different IAM surfaces using a mass spectrometer as a detector and single column chromatography. Note that these compounds have similar retention times and that parallel chromatography that utilizes one detector will unavoidably have all of the compounds co-elute to variable degrees through the common detector. Molecular weight differences provide the needed information to generate chromatograms. Thus, these compounds can be used as solutes for testing the present invention. The experimental design is shown in Fig. 13.

Parallel chromatography generates overlapping peaks in the total ion chromatogram (TIC). One data-file will exist as opposed to the 4 data-files generated by single column chromatography (Fig. 13). Individual chromatograms for each column can be extracted from the single data file. Variable wash cycles set the file format of the data-file as depicted in Fig. 13. For instance, in the partial data-file

house by Finnigan. However, many have been developed by external instrument companies such as Gilson and Beckman through a program called Virtual Instrument Partnership (VIP). Through this program, Finnigan supplies documentation and guidance in writing software that is able to: control instrument parameters, initiate data acquisition, and read the resulting data files from any of their mass spectrometers.

When this driver has been refined and meets all of Finnigan's requirements it is actually incorporated into the next release of their application software. This means that the off-the-shelf version of the software will immediately be able to use the accessory for which the driver was written. According to a contact at Finnigan, no accessory manufacturer has ever been turned down for inclusion in the VIP program.

Detector

The detection device coupled to the chromatographic system is a detector that produces signals/data that allow identification and quantification of detected compounds alone or in mixtures with other compounds. Exemplary of such detectors is a mass spectrometer, preferably run in a mild ionization mode such as electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) and with either magnetic/electric sector or quadrupole rods as mass filters. Other examples of such detectors include those based on infrared or ultraviolet spectroscopy (*e.g.*, FTIR or FTUV) and those based on nuclear magnetic resonance spectroscopy (NMR). Although FTUV technology does not exist yet, it is believed that quality research efforts over the past decades, aimed at developing such technology, have produced significant advances in this field to enable its implementation in the near future.

Examples of detectors other than mass spectrometers that are useful in the present invention are shown in Table 3.

Table 5. Software Tasks

Control-software	Data analysis software
- Variable flow rate pump control	- Peak display from multiple columns
- Binary system pump control	- Mobile phase gradient display from multiple columns
- Variable rate valve control	
- Auto injector control	- Peak characterization (retention times, peak width, etc.)
- Single-Channel UV/VIS detector data acquisition	
	- Curve fitting for under sampled peaks
- Optimization of chromatogram display for a fixed wash cycle	
- Software control of variable wash	

Validation of the present invention was supported by the manufacturing of a prototype four-column ^{HT}HPLC-UV system. The system was built according to the design depicted in Fig. 5, with in-line pH-meters and pressure sensors incorporated in the system. The ^{HT}HPLC-UV is controlled by a dedicated PC through two interface boards. The user interface is programmed in the LabVIEW programming language. The computer system controls valve switching, injector actuation, and pump speed. It also acquires and logs data signals from the detector, pH meters and pressure sensors. All ^{HT}HPLC operating parameters are user-configurable using the custom LabVIEW interface.

Hardware on the ^{HT}HPLC system includes four injectors, and five diaphragm valves. All can be controlled either manually or under computer control through TTL signals. Manual control is through switches on the front panel of the ^{HT}HPLC system. LED's on the front panel indicate the current state of each valve and indicator. The ^{HT}HPLC system also includes four pressure sensors and four pH sensors, one for each column. Outputs from these devices are connected to eight panel

receptor molecule and a compound wherein the receptor molecule includes a defined binding locus that discriminatorily binds those compounds which have a predetermined chemical structure. Compounds not having the predetermined chemical structure do not bind with the binding site of the receptor molecule. "Compound-dependent non-specific binding" as used herein refers to that affinity interaction between a compound and a surface that does not have a specific discriminative binding locus for that compound, but rather the binding derives from the concomitant hydrophobic and/or hydrophilic interactions between the surface and the compound. Non-specific binding between a surface and a compound is "compound-dependent" in that, for any one surface, different compounds will interact and bind with such surface to varying degrees based upon the chemical structure and hydrophobic/hydrophilic nature of the compound. The high sensitivity of the MS detector allows the instantaneous identification of mixtures of compounds.

A mixture of 100 or more compounds can be injected simultaneously on several columns run in parallel and detected as they elute from the chromatographic system. In theory, depending on the loading capacity of the columns, a mixture of up to 1000 compounds can be analyzed simultaneously and in parallel on several chromatographic surfaces. The data from the MS analysis will be correlated to that of the UV detectors connected to each column, resulting in the assignment of a retention time and capacity factor for each and every compound detected. The data can be collected electronically and used as input for the calculation of one or more physicochemical values according to predetermined algorithms or equations.

In summary, the present invention can be applied to the rapid and efficient collection of databases of physicochemical values and/or biologically relevant parameters for large compound libraries. Consequently the present invention seems perfectly suited for lead identification and optimization of chemical libraries, which is a very important aspect of the drug discovery process, as well as QSAR studies. Once a "hit" compound has been identified, derivatization by the usual combinatorial chemistry tools to a large number of structurally similar parent molecules is possible. The present invention provides a convenient and efficient technique for the analysis of this pool of derivatives and the identification of one or more compounds with a data set of physicochemical values (derived from the chromatographic system) that would

control the direction of solvent flow in each column to the detector. These figures illustrate potential methods of implementing the regulation of eluent flow by use of a magnetic switch.

As detailed in Fi. 14, one embodiment of the present invention relates to a modified version of the pre-detector chamber mentioned above whereby four individual compartments (three connected to the columns and the fourth connected to the reference solvent outlet) are mounted directly onto the chamber. Each compartment has an orifice through which the eluent accesses the main chamber. The opening/closing mechanism of the orifice can be accomplished by electromagnetic control of a steel ball (rubber or TEFLON coated) that fits perfectly in the groove of the compartment opening. Activation of the electric magnet removes the ball from the orifice mouth, thus allowing the eluent or wash solution to enter the main chamber. After the magnet is turned off, the steel ball is subjected to both gravity and eluent motion and will return and sit in the orifice cavity to close it. In a similar fashion as for the switch valves described above, the opening/closing of all four compartments can be controlled by computer software interfaced with the system to ensure the alternative delivery of the column eluents as well as the wash solution in the main chamber leading to the electrospray source housing, according to a predetermined sequence.

Two alternative embodiments of this design are illustrated in Figs. 15 and 16. In Fig. 15, the eluent switch is configured with the eluent waste port located upstream (eluent flow-wise) from the steel ball and electomagnet such that, when the electromagnet is activated, the steel ball is moved out of the orifice mouth, thereby allowing eluent to flow into the low volume chamber without restricting eluent flow through the eluent waste port. From the low volume chamber, the eluent flows through a coupling to the sampling port of the detector. In this embodiment, the eluent waste port remains constantly open and at least a portion of the eluent from each respective column is constantly available for collection through the eluent waste port. In this configuration, the continuous collection of sample fractions is possible by using a fraction collector in eluent flow communication with the eluent waste port.

In Fig. 16, another embodiment is demonstrated. In this embodiment, the eluent switch is configured with the eluent waste port located in close proximity to the electromagnets, which optionally are configured to form a magnetic collar

CLAIMS:

1. A chromatographic system comprising:

at least two chromatographic units each having a sample compound loading system, a mobile phase entry port, an eluent exit port, and a stationary phase;

a mobile phase supply system for delivering mobile phase to the mobile phase entry port of each chromatographic unit;

a detector having an eluent sampling port, said detector capable of providing a signal of the presence or identity of an eluted sample compound in an eluent sample delivered to the sampling port;

an eluent switch in eluent flow communication with each of the chromatographic units for delivering aliquots of eluent from each chromatographic unit sequentially to the eluent sampling port on the detector; and

a data management device for receiving or storing signals from the detector.

2. The chromatographic system of claim 1 wherein the mobile phase supply system includes a mobile phase pump for each chromatographic unit.

3. The chromatographic system of claim 2 wherein the pressure generated by the pumps is variable to control the rate of flow of mobile phase in each chromatographic unit.

4. The chromatographic system of claim 1 wherein the sample compound loading system comprises a valve that allows delivery of sample compounds and mobile phase into the chromatographic units when the valve is in one position and allows delivery of only mobile phase into the chromatographic units when the valve is in a second position.

5. The chromatographic system of claim 1 wherein the injector comprises an injector valve and at least two sample loops wherein the injector valve, when placed in one position, allows delivery of sample compounds into the sample loops and also allows the mobile phase to bypass the sample loops and be delivered into the chromatographic units; said injection valve, when placed in another position, allows delivery of sample compounds and mobile phase from the sample loops into the chromatographic units.

eluent portion delivered to the detector;

generating a signal when such presence or identity of an eluted sample compound is detected; and

receiving or storing such signal.

13. The method of claim 12 wherein the mobile phase is supplied using a mobile phase supply system comprising a mobile phase pump for each chromatographic unit.

14. The method of claim 13 wherein the pressure generated by the pumps is varied to control the rate of flow of mobile phase in each chromatographic unit.

15. The method of claim 12 wherein the step of applying the sample compounds into at least two chromatographic units is accomplished through using an injector comprised of a valve that allows delivery of sample compounds and mobile phase into each of the chromatographic units when the valve is in one position and allows delivery of only mobile phase into each of the chromatographic units when the valve is in a second position.

16. The method of claim 12 wherein the step of applying the sample compounds into at least two chromatographic units is accomplished through using an injector comprised of a valve and at least two sample loops wherein the injector valve, when placed in one position, allows delivery of the compounds into the sample loops and also allows the mobile phase to bypass the sample loops and be delivered into the chromatographic units; said sample valve, when placed in a second position, allows delivery of the compounds and mobile phase from the sample loops into the chromatographic units.

17. The method of claim 12 wherein the step of detecting the presence or identity of an eluted compound is accomplished by using a detector comprised of a mass spectrometer, a Fourier transform infra red spectrometer, a Fourier transform ultra violet spectrometer, or a Fourier transform nuclear magnetic resonance spectrometer.

18. The method of claim 17 wherein the detector is a mass spectrometer.

19. The method of claim 12 wherein the step of eluting the sample

respective chromatographic unit as a function of time or volume of eluent.

26. A method for data acquisition by analysis of eluent streams from multiple chromatographic columns in a chromatographic system using an eluent stream analyzer having a fluid sampling port and capable of providing signals characteristic of detected chemical species in an eluent stream, said method comprising

providing an eluent switching valve having

(1) fluid input ports in fluid communication with the eluent stream from each chromatographic column and with a source of at least one indexing fluid,

(2) a fluid output port in fluid flow communication with a fluid sampling port on the eluent stream analyzer, and

(3) a valve system communicating with a programmable controller for directing aliquots of the respective eluent streams and indexing fluid in a programmed sequence through the fluid output port and toward the fluid sampling port on the eluent stream analyzer, and

correlating the electronic storage of signals from the analyzer with the programmed sequence so that analyzer signals from analysis of the aliquots of eluent of each respective column are stored in an algorithm accessible electronic storage device.

27. The method of claim 26 wherein the correlation of the electronic storage of signals from the analyzer include the step of sensing aliquots of indexing fluid between each eluent stream aliquot.

28. The method of claim 26 wherein the indexing fluid comprises a liquid.

29. The method of claim 28 wherein the liquid comprises at least one analyzer detectable species.

30. The method of claim 26 wherein the indexing fluid comprises a gas.

31. The method of claim 26 wherein the indexing fluid comprises a liquid and a gas.

32. The method of claim 26 wherein the chromatographic system further comprises a pump for controlling pressure or a valve for controlling rate of flow of the respective eluent streams and indexing fluids through the valve system and

an electronic data storage device for receiving and storing signals from the eluent stream analyzer representative of analysis of eluent stream aliquots of each respective chromatographic column; and

a programmable controller (computer) including an algorithm including instructions for sensing the presence, volume or components of an indexing fluid aliquot at the fluid sampling port of the analyzer and directing signals from the analyzer for eluent stream aliquots corresponding to the respective chromatographic columns to electronic data storage registers/devices designated for data storage for the respective chromatographic columns.

38. The multi-column chromatographic system of claim 37 wherein the fluid input port on the eluent switching valve assembly is positioned for fluid communication with the eluent stream from each chromatographic column and with a pressure control source of at least one indexing liquid and at least one indexing gas.

39. The multi-column chromatographic system of claim 37 wherein the eluent stream analyzer comprises a mass spectrometer.

40. The multi-column chromatographic system of claim 37 wherein the eluent stream analyzer comprises an infra-red spectrometer.

41. The multi-column chromatographic system of claim 37 wherein the eluent stream analyzer comprises a nuclear magnetic resonance spectrometer.

42. The multi-column chromatographic system of claim 37 wherein the eluent stream analyzer comprises an ultraviolet-visible spectrometer.

43. The multi-column chromatographic system of claim 37 wherein the eluent stream analyzer is selected from the group consisting of a fluorescence detector, an electrochemical detector and a refractive index detector.

44. The multi-column chromatographic system of claim 26 wherein the eluent stream analyzer is selected from the group consisting of a mass spectrometer, an infra-red spectrometer, a nuclear magnetic resonance spectrometer, an ultraviolet-visible spectrometer, a fluorescence detector, an electrochemical detector and a refractive index detector.

45. The method of claim 26 wherein the signal from the analyzer is used as "feed back" signal to control the programmable controller.

46. The method of claim 37 wherein the signal from the analyzer is

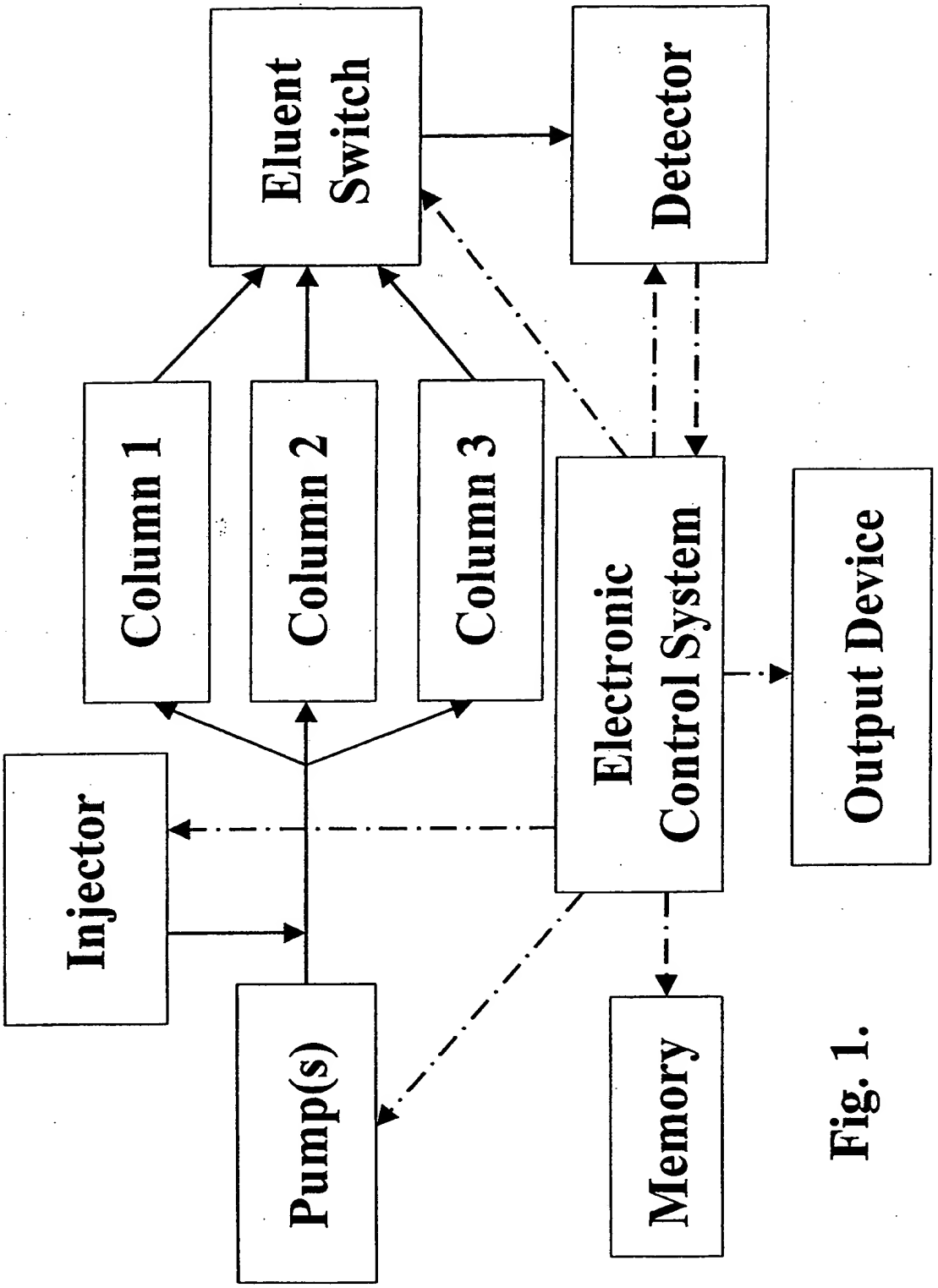
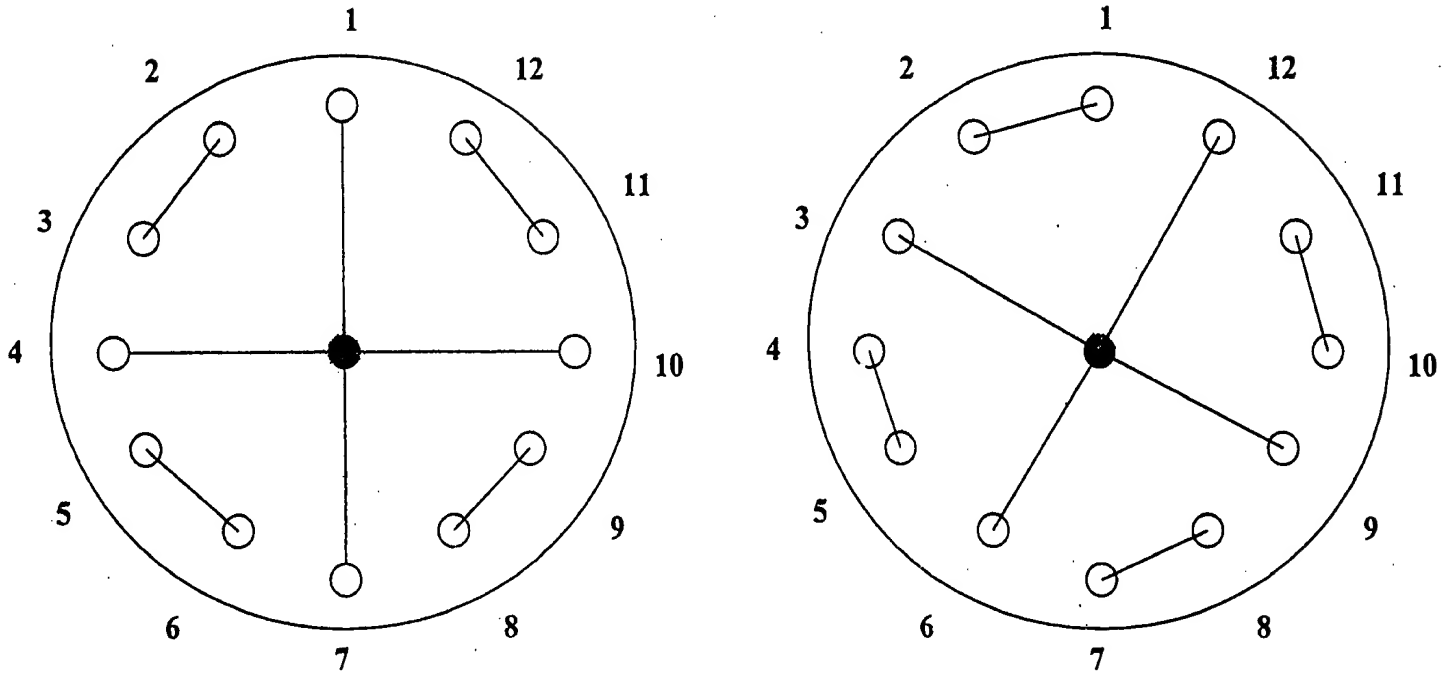

Eluent Switch
Detector
Column 1
Column 2
Column 3
Electronic Control System
Output Device
Injector
Pump(s)
Memory

Fig. 1.

Fig. 3.



Position: **1** **2**

Loading Configuration: A (1), B (1)
Injecting Configuration: A (2), B (2)

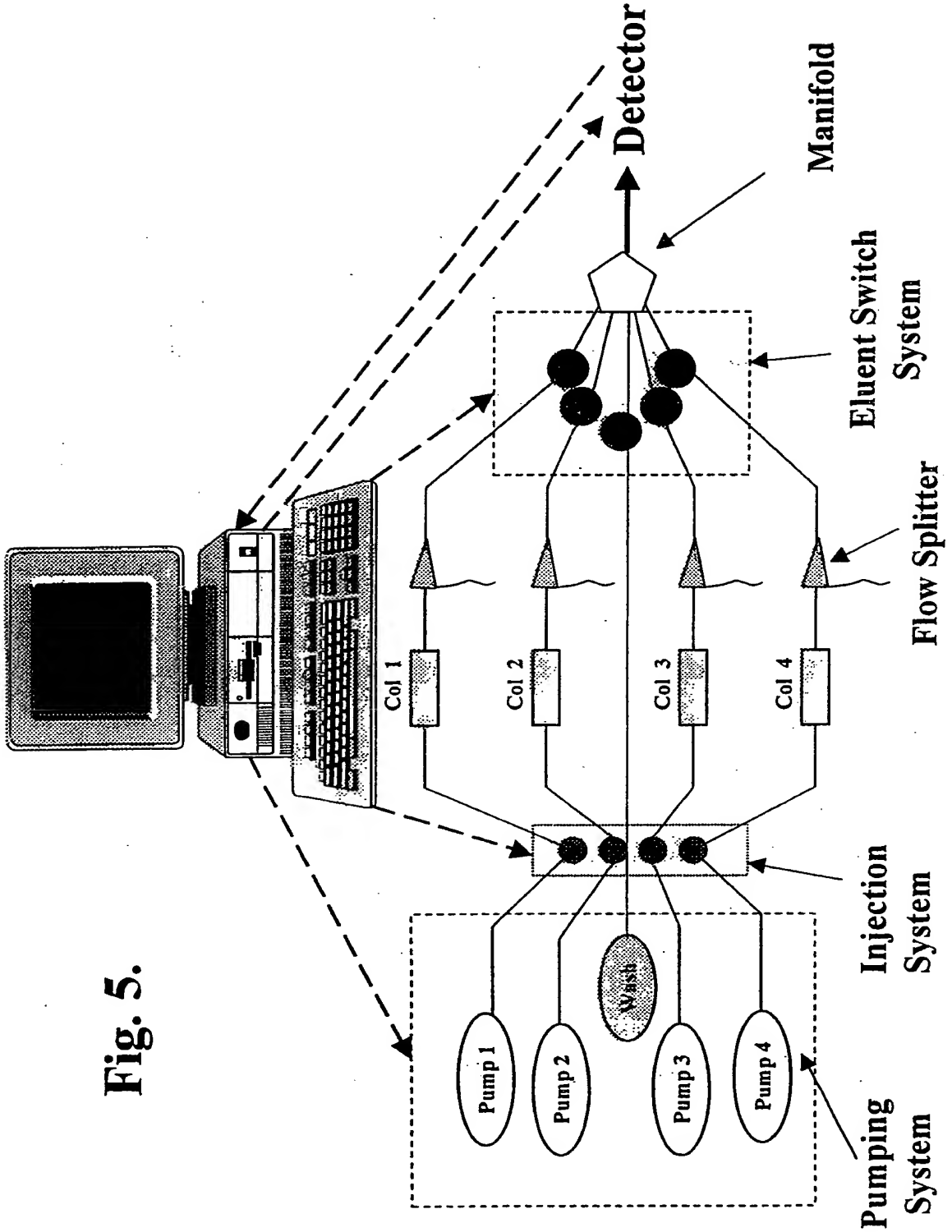

Detector
Manifold
Eluent Switch System
Flow Splitter
Col 1
Col 2
Col 3
Col 4
Injection System
Wash
Pump 1
Pump 2
Pump 3
Pump 4
Pumping System

Fig. 5.

Fig. 7.

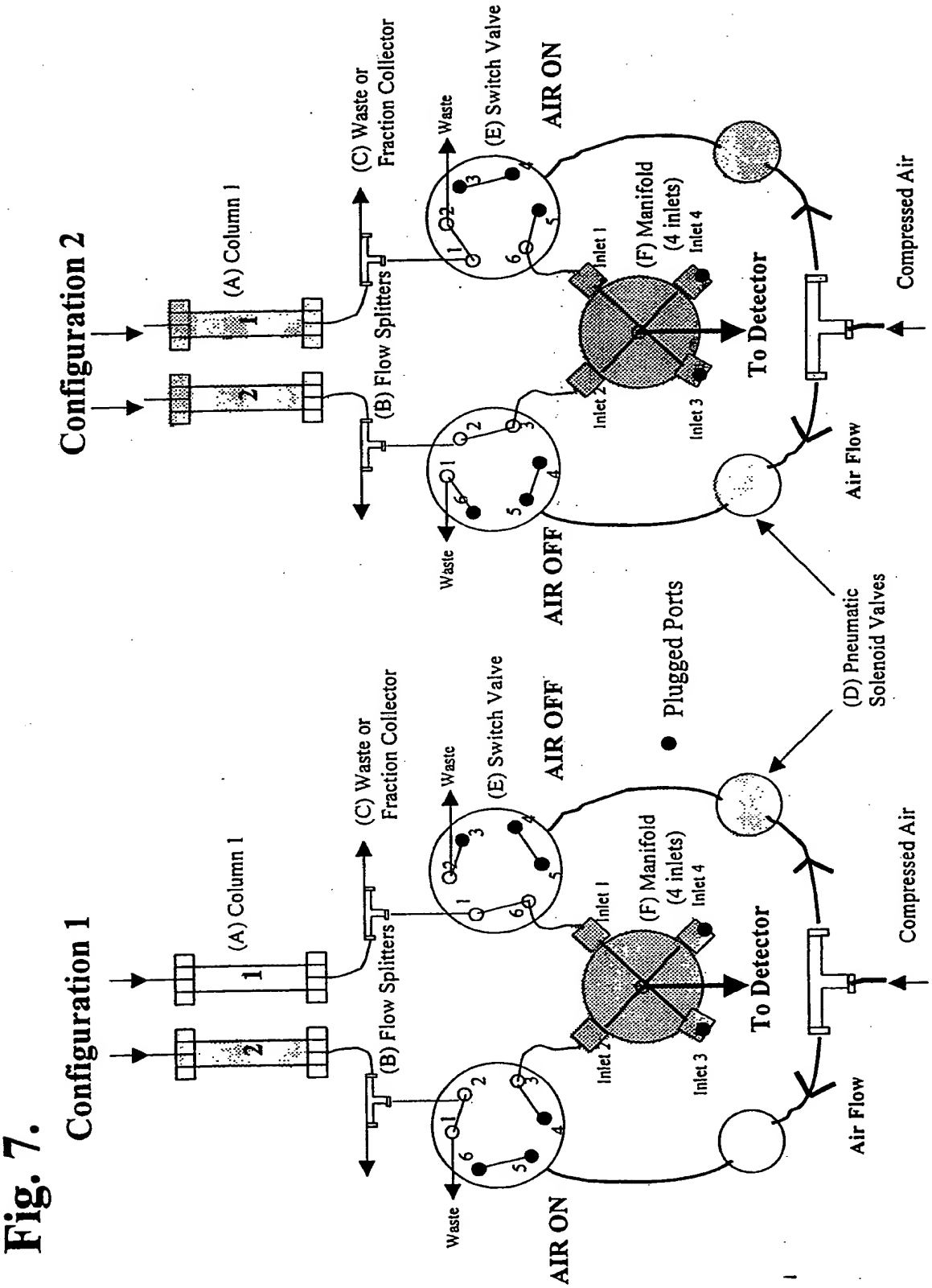
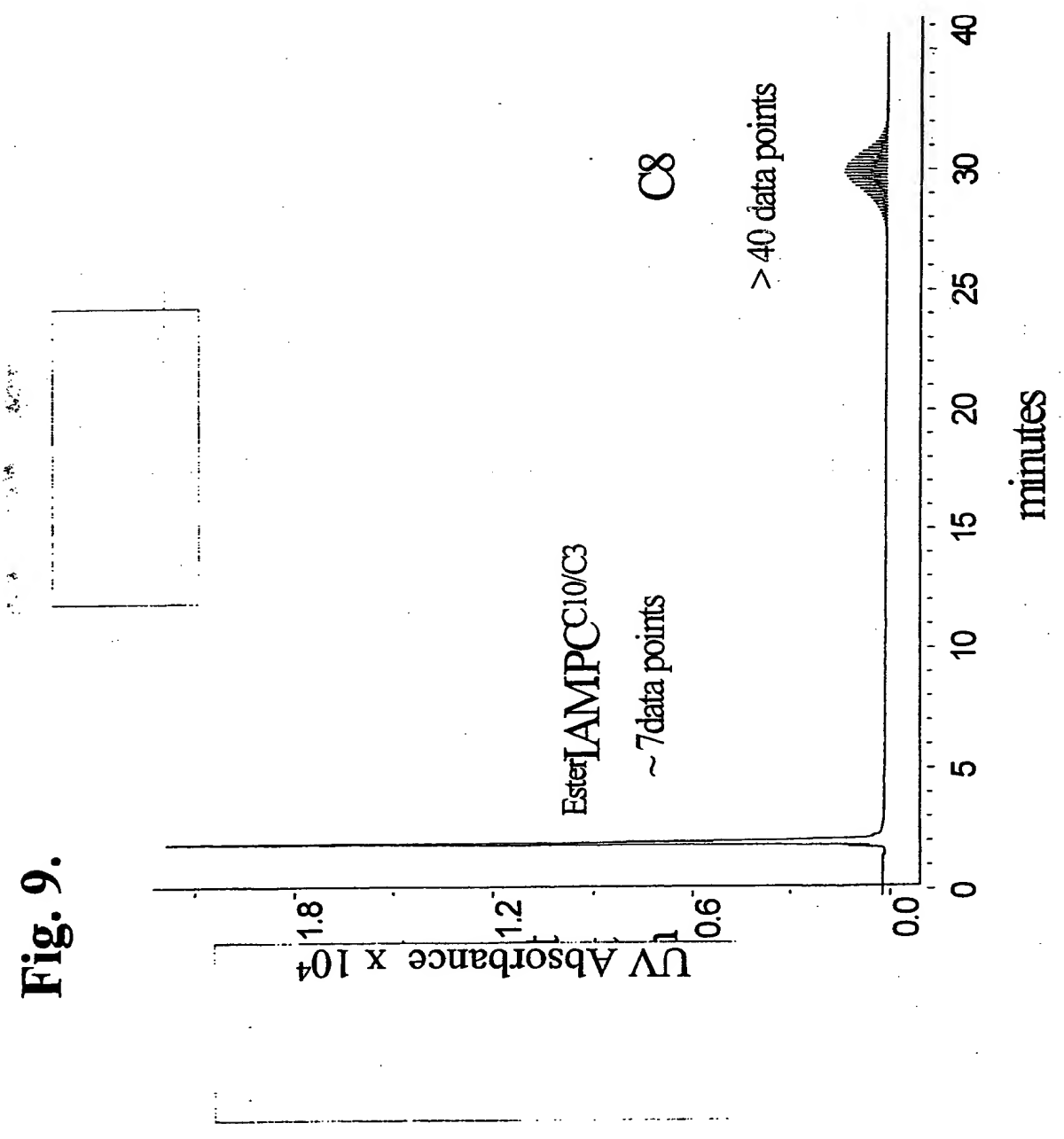

Configuration 1
(A) Column 1
(B) Flow Splitters
(C) Waste or Fraction Collector
(E) Switch Valve
Waste
AIR ON
AIR OFF
(F) Manifold (4 inlets)
Inlet 1
Inlet 2
Inlet 3
Inlet 4
To Detector
Air Flow
Compressed Air
(D) Pneumatic Solenoid Valves
Plugged Ports
Configuration 2
(A) Column 1
(B) Flow Splitters
(C) Waste or Fraction Collector
(E) Switch Valve
Waste
AIR ON
AIR OFF
(F) Manifold (4 inlets)
Inlet 1
Inlet 2
Inlet 3
Inlet 4
To Detector
Air Flow
Compressed Air

Fig. 9.


UV Absorbance x 10⁴
1.8
1.2
0.6
0.0
0
5
10
15
20
25
30
40
minutes
EsterLAMPC C10/C3
~7data points
C8
>40 data points

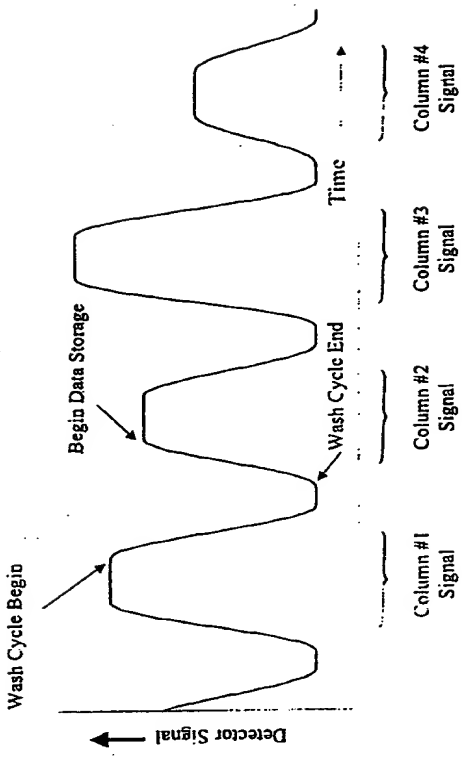
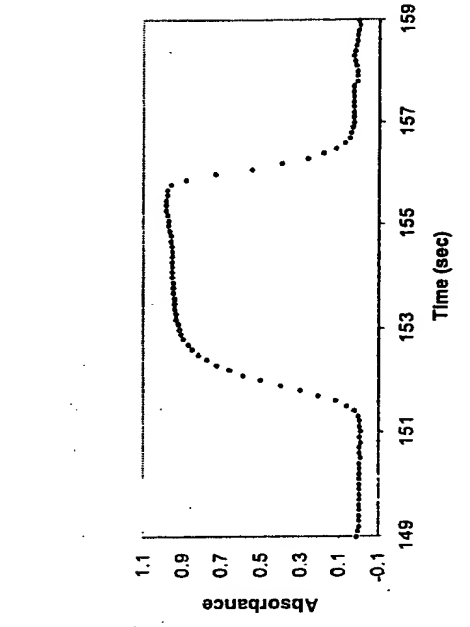


Fig. 11.

Fig. 11A



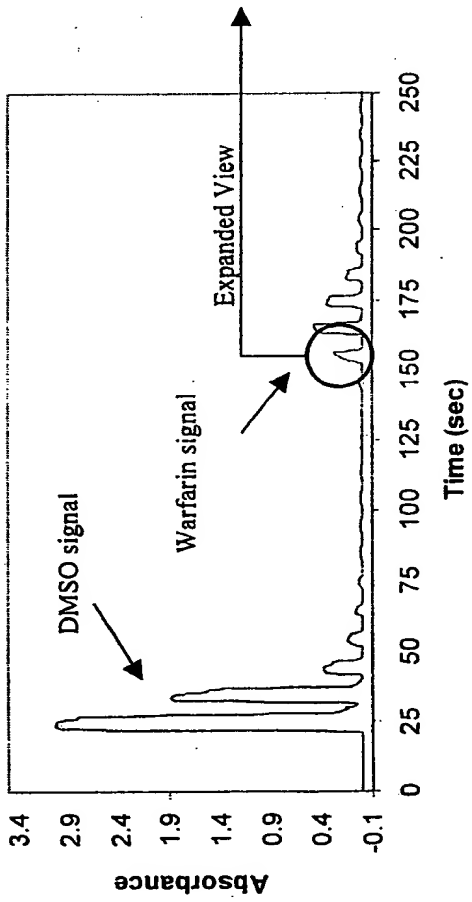


Fig. 11B

Fig. 13.

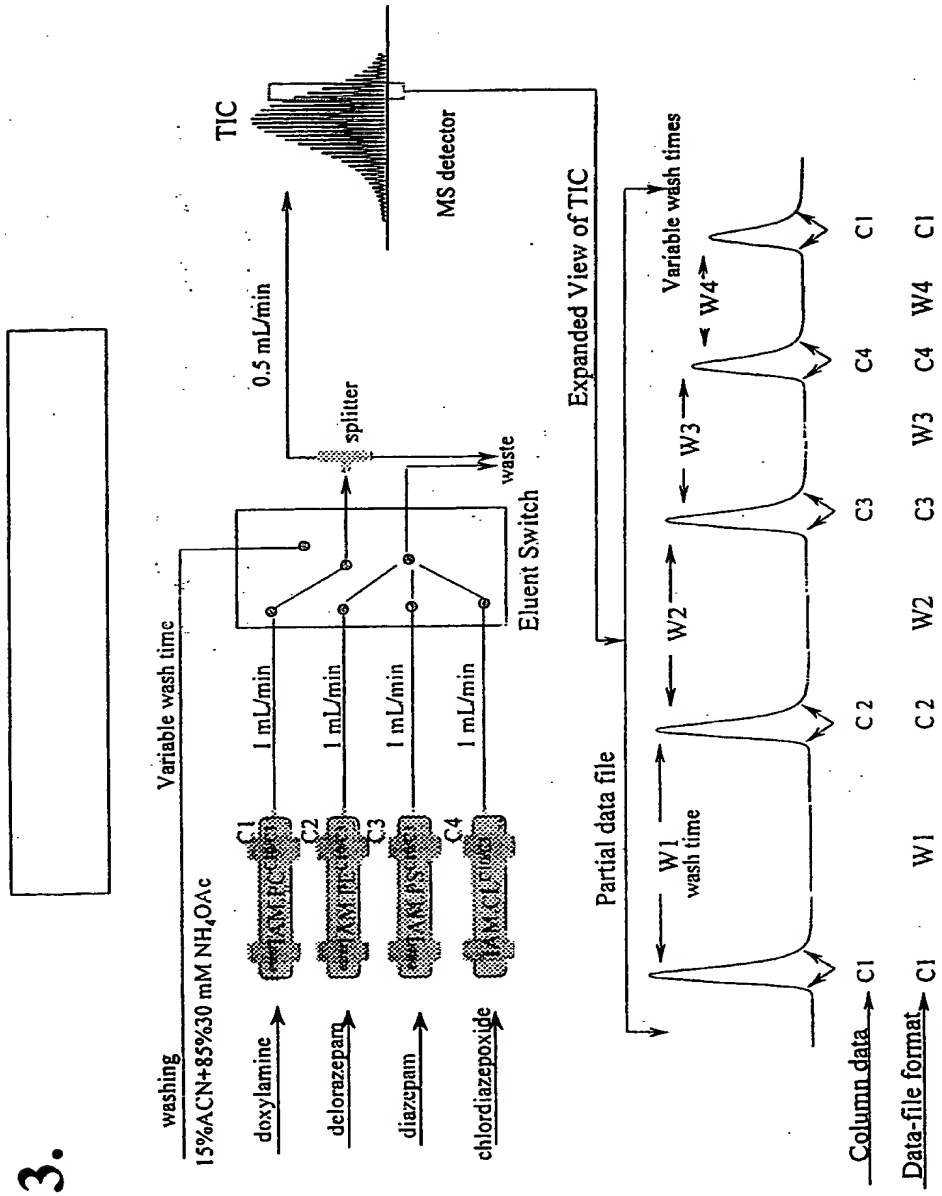
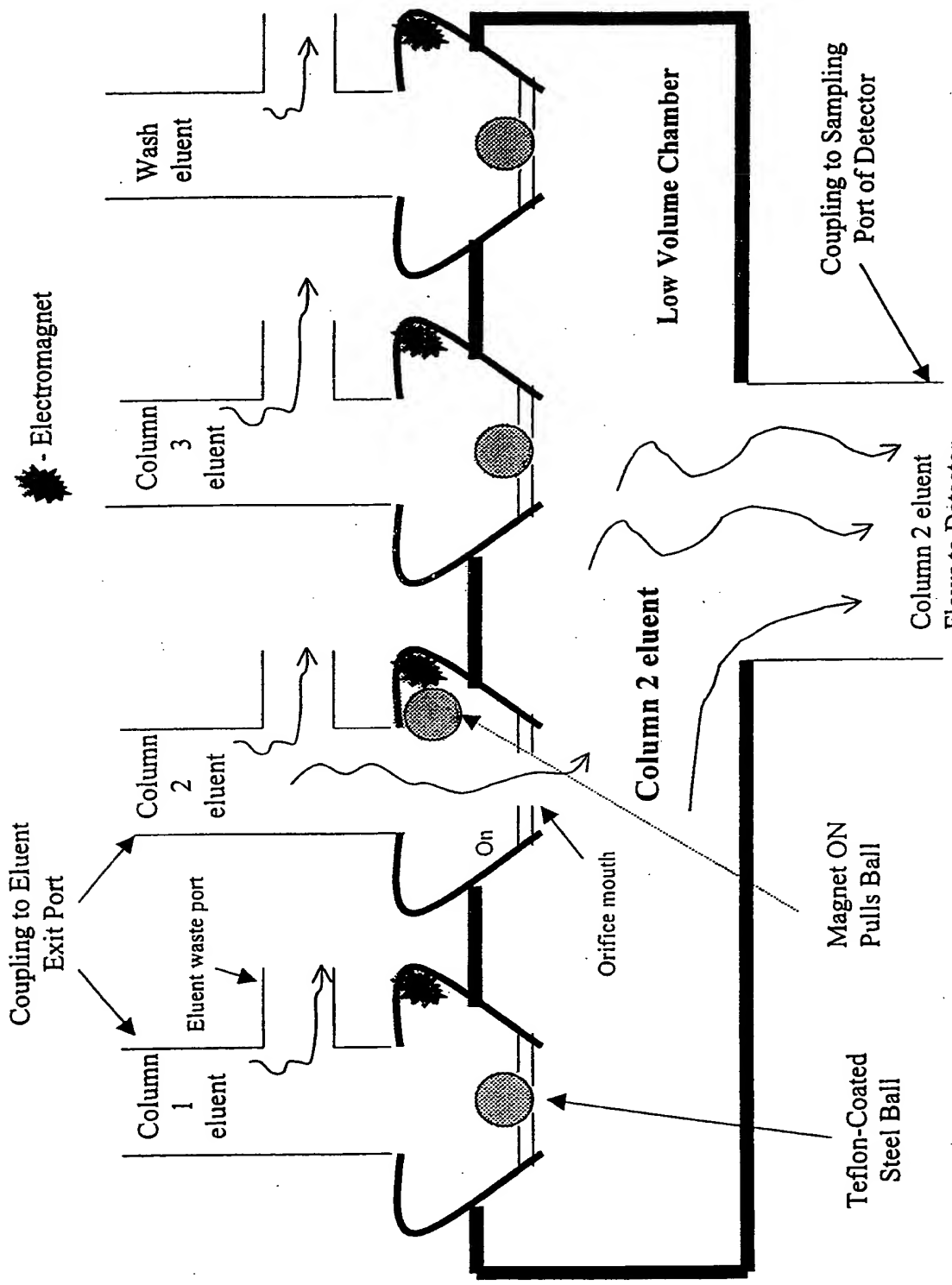

washing
15%ACN+85%30 mM NH_4OAc
Variable wash time
doxylamine
delorazepam
diazepam
chlordiazepoxide
1 mL/min
1 mL/min
1 mL/min
1 mL/min
C1
C2
C3
C4
Eluent Switch
waste
splitter
0.5 mL/min
TIC
MS detector
Expanded View of TIC
Partial data file
W1
wash time
W2
W3
W4
Variable wash times
Column data
Data-file format
C1 W1 C1
C2 W2 C2
C3 W3 C3
C4 W4 C4
C1 C1

Fig. 15.


Coupling to Eluent Exit Port
Column 1 eluent
Eluent waste port
Column 2 eluent
Column 3 eluent
Wash eluent
- Electromagnet
On
Orifice mouth
Column 2 eluent
Low Volume Chamber
Coupling to Sampling Port of Detector
Column 2 eluent Flows to Detector
Magnet ON Pulls Ball
Teflon-Coated Steel Ball

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To:
BROMBERG & SUNSTEIN LLP
Attn. Smolenski, Alexander J.
125 Summer Street
Boston, Massachusetts 02110-1618
UNITED STATES OF AMERICA

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

	Date of mailing *(day/month/year)* 27/09/2002
Applicant's or agent's file reference 2119/104WO	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US 02/ 17438	International filing date *(day/month/year)* 04/06/2002
Applicant BIOTROVE, INC.	

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gregory Adam

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the International application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

NOTES TO FORM PCT/ISA/220 (continued)

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicat , in connection with ach claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),wheth r

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments and any accompanying statement, under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the time of filing the amendments (and any statement) with the International Bureau, also file with the International Preliminary Examining Authority a copy of such amendments (and of any statement) and, where required, a translation of such amendments for the procedure before that Authority (see Rules 55.3(a) and 62.2, first sentence). For further information, see the Notes to the demand form (PCT/IPEA/401).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
2119/104WO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 02/ 17438	04/06/2002	27/06/2001
Applicant		
BIOTROVE, INC.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 2 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 2

   [X] as suggested by the applicant.

   [ ] None of the figures.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 02/17438

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: B01D 15/08, G01N 30/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: B01D, G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPODOC, WPI, PAJ, DIALINDEX:ALLSCIENCE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 0045929 A1 (CPBD, INC.), 10 August 2000 (10.08.00), page 45, line 26 - page 46, line 15, figure 5	1,20

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☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
18 Sept 2002

Date of mailing of the international search report
27.09.02

Name and mailing address of the International Searching Authority
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NL-2280 HV Rijswijk
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INTERNATIONAL SEARCH REPORT

Information on patent family members

02/09/02

International application No.
PCT/US 02/17438

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0045929 A1	10/08/00	AU 2986400 A	25/08/00